

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

OFFICE OF  
PREVENTION, PESTICIDES  
AND TOXIC SUBSTANCES

PC Code No.: 041101

DP Barcode: 306508

Date: October 30, 2007

**MEMORANDUM**

**SUBJECT:** Data Evaluation Record for MRID 46315401: "Ethoprop - Chronic Toxicity to the Sheepshead Minnow (*Cyprinodon variegatus*) During a Full Life-Cycle Exposure."

**TO:** Michael Goodis, Branch Chief  
Reregistration Branch III  
Special Review and Reregistration Division (7508P)

**FROM:** Michael D. Hoffmann, Biologist  
Environmental Risk Branch 5  
Environmental Fate and Effects Division (7507P)

**THROUGH:** Mah Shamim, Branch Chief  
Environmental Risk Branch 5  
Environmental Fate and Effects Division (7507P)

Attached to this memo you will find the file containing the Data Evaluation Record (DER) MRID 46315401: "Ethoprop - Chronic Toxicity to the Sheepshead Minnow (*Cyprinodon variegatus*) During a Full Life-Cycle Exposure." This study is classified as **INVALID** and does not fulfill guideline requirements (§72-5) for estuarine/marine fish full life-cycle toxicity tests because it was performed under conditions that deviated so significantly from recommended protocols that the results are not useful for risk assessment purposes. Among other deviations, an inappropriate number of replicates are used and the ability of this study to detect significant treatment effects is compromised. In addition, guideline requirements specify that the hatching success of the controls for sheepshead minnows should be >75%; however, the mean hatching success of the controls in this study is 57% (page 27 of the report). Subsequently, the ability of this study to detect treatment-related effects on this endpoint may be compromised and it is highly uncertain how overall poor hatching success of the source population for test subjects may influence the estimation of other endpoints. Therefore, EFED recommends that a new full life-cycle study establishing a reliable NOAEC for estuarine/marine fish be conducted.

Table 1. Reference information for the attached toxicity study.					
Guideline #	Data Requirement	Test Substance (Purity)	MRID	Study Classification	Are additional data needed for ecological risk assessment?
72-5	Full-life Cycle Study With Exposure/Marine Fish	TGAL (94.8%)	61315411	Acute	Yes

**DATA EVALUATION RECORD  
FISH LIFE-CYCLE TOXICITY TEST  
§72-5**

1. **CHEMICAL:** Ethoprop **PC Code No.:** 041101

2. **TEST MATERIAL:** Ethoprophos Technical **Purity:** 94.8% (w:w)

3. **CITATION:**

**Author:** Dionne, E.

**Title:** Ethoprop - Chronic Toxicity to the Sheepshead Minnow  
(*Cyprinodon variegatus*) During a Full Life-Cycle  
Exposure.

**Study Completion Date:** June 29, 2004

**Laboratories:** Springborn Smithers Laboratories  
790 Main Street  
Wareham, MA 02571-1075

**Sponsor:** Bayer CropScience  
17745 South Metcalf Avenue  
Stilwell, KS 66085

**Laboratory Report ID:** 13798.6154

**MRID No.:** 46315401

**DP Barcode:** D306508

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, Dynamac Corporation

**Signature:**

**Date:** 11/23/04

**APPROVED BY:** Teri Myers, Ph.D., Staff Scientist, Dynamac Corporation

**Signature:**

**Date:** 12/7/04

5. **APPROVED BY:** Michael Hoffmann, OPP/EFED/ERB-V

**Signature:**



**Date:** 10/30/07

**DATA EVALUATION RECORD  
FISH LIFE-CYCLE TOXICITY TEST  
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**Signature:** 

**Date:** 12/7/04

5. **APPROVED BY:** Allen Vaughan, OPP/EFED/ERB-V

**Signature:**

**Date:**

**6. STUDY PARAMETERS:**

**Scientific Name of Test Organism:** *Cyprinodon variegatus*

**Age of Test Organism:** Embryos, >24 to <48 hours old (F<sub>0</sub> generation)

**Definitive Test Duration:** 112 Days (Approx. 3.5 months)

**Study Method:** Flow-through

**Type of Concentrations:** Mean-measured

**7. CONCLUSIONS:**

The 3.5-month chronic toxicity of Ethoprophos Technical (ethoprop) to the full life stage of Sheepshead minnow (*Cyprinodon variegatus*) was studied under flow-through conditions. Fertilized eggs (280 embryos/treatment, >24 to <48 hours old) were exposed to the test material at nominal concentrations of 0 (negative control), 1.0, 2.0, 4.0, 8.0, and 16 ppb a.i. Mean-measured concentrations were <0.22 (control), 1.2, 2.0, 3.7, 7.4, and 15 ppb a.i., respectively, and were generally stable during the study.

Following the completion of hatching on Day 6, larvae were reduced to 100 per treatment level. At 4-weeks post-hatch, the juveniles were again reduced to 50 per treatment level. Spawning was initiated 8 weeks following hatching: four groups of two male and five female per test level were assigned to spawning aquaria, and hatchability trials and early life stage studies were performed for the F<sub>1</sub> generation. Following hatching, the F<sub>1</sub> generation was maintained for 4 weeks. The F<sub>0</sub> portion of test was terminated 16 weeks after hatching (Day 118).

This study is classified as **INVALID** and does not fulfill guideline requirements (§72-5) for estuarine/marine fish full life-cycle toxicity tests because it was performed under conditions that deviated so significantly from recommended protocols that the results are not useful for risk assessment purposes. Among other deviations, an inappropriate number of replicates are used and the ability of this study to detect significant treatment effects is compromised.

In addition, guideline requirements specify that the hatching success of the controls for sheepshead minnows should be >75%; however, the mean hatching success of the controls in this study is 57% (page 27 of the report). Subsequently, the ability of this study to detect treatment-related effects on this endpoint may be compromised and it is highly uncertain how overall poor hatching success of the source population for test subjects may influence the estimation of other endpoints. Therefore, EFED recommends that a new full life-cycle study establishing a reliable NOAEC for estuarine/marine fish be conducted.

**Results Synopsis:****NOEC:** Invalid study**LOEC:** Invalid study**MATC:** Invalid study**Endpoint(s) Affected:** Invalid study**8. ADEQUACY OF THE STUDY:****A. Classification:** Invalid

**B. Rationale:** The study deviated so significantly from guideline recommendations, that the study cannot be used for risk assessment purposes. In particular, an inappropriate number of replicates are used and the ability of this study to detect significant treatment effects is compromised. In addition, guideline requirements specify that the hatching success of the controls for sheepshead minnows should be >75%; however, the mean hatching success of the controls in this study (57%; page 27 of the study report) does not meet guideline requirements. Subsequently, the ability of this study to detect treatment-related effects on this endpoint may be compromised and it is highly uncertain how overall poor hatching success of the source population for test subjects may influence the estimation of other endpoints.

**C. Repairability:** This study is not upgradable and does fulfill the guideline requirement for a full life-cycle toxicity test (§72-5).

**9. GUIDELINE DEVIATIONS:**

1. The temperature ranged from 24-27°C throughout the study, which is less than the recommended temperature of 30°C for this species.
2. It was not reported if aeration of the test aquaria was necessary during the study.
3. F<sub>1</sub>-generation fish were maintained for only 4 weeks, instead of the required 8 weeks.
4. The reviewer-calculated high-low ratio of mean-measured concentrations was 1.7 for the nominal 2.0 ppb treatment level, exceeding the 1.5 limit.
5. This study design only included 2 true replicates, despite that guidelines recommend at least 4 replicates for determining hypothesis based endpoints.

Subsequently, the ability of this study to detect significant treatment effects is compromised.

6. Control performance in fish life-cycle studies should meet the same standards as set forth in the OPPTS guideline 850.1400 for fish early life-stage toxicity studies. In particular, 850.1400 guideline requirements specify that the hatching success of the controls for sheepshead minnows should be >75%; however, the mean hatching success of the controls in this study (57%; page 27 of report) does not meet guideline requirements. Subsequently, the ability of this study to detect treatment-related effects on this endpoint may be compromised and it is not clear whether the most sensitive NOAEC value reported in this study is in fact the most sensitive endpoint for this test species. In addition, it is highly uncertain how overall poor hatching success of the source population for test subjects may influence the estimation of other endpoints.
7. A description of the approach and criteria by which the viability of the source eggs were estimated were not provided for evaluation, nor were raw data for the estimate of 68% viability. Although the data should not have been adjusted to begin with because the recommended study design dictates that while "abnormal embryos and larvae occur naturally" they should only be "of the order of several percent in the controls," this information should have been reported since the decision was made to adjust all % hatch data for all test concentrations based on an estimated mean viability of eggs of 68%. In addition, the overall validity of the test is dependent upon maintaining the standards set in OPPTS guideline 850.1400 for percent hatch and survival of fertilized eggs in the controls, as specified above.

**10. SUBMISSION PURPOSE:** This study was submitted to provide data on the toxicity of ethoprop to the full life-cycle of sheepshead minnows for the purposes of chemical re-registration.

**11. MATERIALS AND METHODS:**

**A. Test Organisms**

Guideline Criteria	Reported Information
<u>Species</u> Prefer Sheepshead minnow ( <i>Cyprinodon variegatus</i> ) or Fathead minnow	Sheepshead minnow ( <i>Cyprinodon variegatus</i> )

Guideline Criteria	Reported Information
<i>(Pimephales promelas).</i>	
<b><u>Source and Acclimation</u></b>	Embryos were obtained from Aquatic Biosystems, Fort Collins, CO. The embryos were held in natural filtered seawater at 22°C for approx. 1 hour, then at 25°C until assignment.
<b><u>Age at beginning of test</u></b> Embryos, 2 to 24 hours old	Embryos, >24 to <48 hours old
<b><u>Feeding</u></b> Fish should be fed at least twice daily and should not be fed for at least 24 hours prior to test termination.	F <sub>0</sub> and F <sub>1</sub> larvae were fed live brine shrimp nauplii three times daily during the first 28 days post-hatch. After 14 days post-hatch, small amounts of dry flaked food was also offered daily. The juvenile and adult fish were fed frozen brine shrimp and Zeigler Prime Flakes twice daily. Feedings were withheld 24 hours before weight determinations.
<b><u>Embryo Exposure (4 to 5 Days)</u></b> Embryos (≤24 hours old) from at least 3 separate spawns should be randomly distributed to embryo cups.  A minimum of 50 embryos (≤24 hrs old) per replicate cup, 4 cups per treatment should be used.  <b><u>Parameters measured:</u></b> <ul style="list-style-type: none"> <li>• Survival of embryos</li> <li>• Time required to hatch</li> <li>• Hatching success</li> <li>• Survival of fry for 4 weeks</li> </ul> Dead and fungused embryos should be counted and removed daily.	<b><u>Days 0-6</u></b> Embryos (> 24 to <48 hours old) were randomly assigned into embryo incubation cups; the number of origin spawns was not reported.  Each cup contained 70 embryos, with two cups per replicate and two replicate aquaria per treatment level (total of 280 embryos per treatment).  <b><u>Parameters measured:</u></b> <ul style="list-style-type: none"> <li>• Hatching success</li> <li>• Time to hatch</li> <li>• Survival of fry at 4 weeks post-hatch</li> </ul> Mortality was determined daily. Dead embryos were removed.



Guideline Criteria	Reported Information
<p><b><u>Larval-Juvenile Exposure (From Hatch to 8 Weeks)</u></b></p> <p>After hatching, each group of larvae is randomly reduced to a minimum of 25 fish and released in replicate larval growth chambers. The random selection must include any fish that are lethargic or deformed.</p> <p><b><u>Parameters measured:</u></b></p> <ul style="list-style-type: none"> <li>· Fish survival (determined by counting the number of live fish in each replicate growth chamber weekly).</li> <li>· Total lengths (mm) of all fish at 4 and 8 weeks after hatching.</li> </ul>	<p><b><u>Hatch to 8 Weeks Post-Hatch</u></b></p> <p>When hatching was complete (on Day 6), 25 larvae were impartially selected from each cup and transferred to the larval growth chambers (two chambers within each replicate aquarium, 100 larvae per treatment). At 4 weeks post-hatch, juvenile fish from the two growth chambers were combined and impartially reduced to 25 per replicate (50 per treatment).</p> <p><b><u>Parameters measured:</u></b></p> <ul style="list-style-type: none"> <li>· Survival of fry/juvenile fish at 8 weeks post-hatch</li> <li>· Total lengths (mm) of all surviving fish at 4 and 8 weeks post-hatch (gender-specific at 8 weeks).</li> <li>· Wet weights (mg) of fish discontinued from exposure (at thinning) at 4 weeks post-hatch</li> </ul>
<p><b><u>Juvenile-Adult Exposure (From 8 weeks posthatch to the end of the spawning phase [32-40 weeks])</u></b></p> <p>At 20-24 weeks after hatching, mature fish are placed in a spawning tank of the same concentration (4 males and 4 females randomly chosen and assigned). The spawning tank is divided into 4 individual spawning chambers with appropriate spawning substrates.</p> <p>The substrates are examined daily and embryos removed, counted, and recorded separately for each pair.</p> <p>For fathead minnow, adult exposure</p>	<p><b><u>8 to 16 Weeks Post-Hatch</u></b></p> <p>Two spawning groups (2 male and 5 female per group) were established for each replicate aquarium. The first group was established on Day 61 (55 days post-hatch), and the second 14 days following the first. Females killed by male aggression were not replaced; however, males were replaced in order to maximize egg fertilization success.</p> <p>The spawning substrates are examined daily and embryos removed, counted, and examined for fertility.</p> <p>Adult exposure was terminated on Day 118 (112 days post-hatch).</p>

Guideline Criteria	Reported Information
<p>should be terminated when no spawning occurs for one week. For sheepshead minnow, testing should be terminated after spawning is observed for 2 weeks.</p>	<p><u>Parameters measured:</u></p> <ul style="list-style-type: none"> <li>• Survival of adult fish</li> <li>• Fecundity (eggs/female/day)</li> <li>• Total lengths (mm) and wet weights (g) of all surviving fish at Day 118 (gender-specific)</li> </ul>
<p><b><u>Second Generation Embryo Exposure (4 to 5 days)</u></b></p> <p>50 embryos from each conc. level are randomly selected and transferred to incubation cups for hatch. Use the same test procedures as those for parental generation.</p> <p>Embryos not selected are discarded.</p>	<p><b><u>F<sub>1</sub> Embryo Exposure</u></b></p> <p>50 embryos from spawns of <math>\geq 50</math> eggs were incubated in each incubation cup as previously described (200 embryos per treatment level).</p>
<p><b><u>Second Generation Larval-Juvenile Exposure (From Hatch to 4-8 weeks)</u></b></p> <p>After hatching, 25 larvae are released in each growth chambers (2 chambers per treatment).</p> <p>Each group of 2<sup>nd</sup> generation fish is terminated 8 weeks after hatching.</p> <p>Fish are blotted, weighed, and measured before being discarded.</p>	<p><b><u>F<sub>1</sub> Larval-Juvenile Exposure</u></b></p> <p>Groups of 25 newly-hatched larvae were randomly released into each larval growth chamber (100 larvae per treatment level).</p> <p>Each group of F<sub>1</sub>-generation fish was terminated 28 days after hatching.</p> <p>Fish were weighed (wet) and measured for total length.</p>

Comments: 850.1400 guideline requirements specify that the hatching success of the controls for sheepshead minnows should be  $>75\%$ ; however, the mean hatching success of the controls in this study (57%; page 27 of report) does not meet guideline requirements. A sub-sample of 100 embryos was examined for viability, and based on visible developmental stages, it was estimated that 68% of the eggs were viable (p. 18). *Embryo hatching success values were adjusted by the study authors using this estimated percent viability.*

## B. Test System

Guideline Criteria	Reported Information
<p><b><u>Test Water</u></b>  <b><u>Sheepshead Minnow</u></b></p> <ol style="list-style-type: none"> <li>1. Natural seawater (sterilized and filtered) or a commercial mixture.</li> <li>2. Natural seawater with a salinity of <math>\geq 15</math> parts per thousand (weekly range of salinity <math>&lt; 6\%</math> and monthly pH range <math>&lt; 0.8</math> pH units).</li> </ol> <p><b><u>Fathead Minnow</u></b></p> <ol style="list-style-type: none"> <li>1. Reconstituted water or water from unpolluted well or spring (sterilized and tested for pollutants).</li> <li>2. Hardness of 40 to 48 mg/L as <math>\text{CaCO}_3</math> and pH of 7.2 to 7.6.</li> </ol>	<ol style="list-style-type: none"> <li>1. Natural filtered seawater collected from Cape Cod Canal, Bourne, MA.</li> <li>2. Salinity of 31-33 ‰ and pH 7.6-8.0.</li> </ol> <p>N/A</p>
<p><b><u>Test Temperature</u></b>  <b><u>Sheepshead:</u></b> 30°C.</p> <p><b><u>Fathead:</u></b> 25°C and should not remain outside the range of 24 to 26°C for more than 48 hours.</p>	<p>24-27°C</p> <p>N/A</p>
<p><b><u>Photo-period</u></b>  16-hour light/8-hour dark.</p> <p>Light intensity of 10-100 lumens at water surface.</p>	<p>16-hour light/8-hour dark cycle</p> <p>Light intensity of 52-116 foot-candles (560-1200 lux)</p>
<p><b><u>Dosing Apparatus</u></b></p> <ol style="list-style-type: none"> <li>1. Intermittent flow proportional diluters or continuous flow serial diluters.</li> <li>2. A minimum of 5 toxicant concentrations with a dilution factor <math>\leq 0.5</math>.</li> <li>3. One control should be used.</li> </ol>	<ol style="list-style-type: none"> <li>1. Intermittent-flow proportional diluter.</li> <li>2. Five toxicant concentrations with a dilution factor of 0.5.</li> <li>3. A dilution water (negative) control was used.</li> </ol>

Guideline Criteria	Reported Information
<p><b><u>Toxicant Mixing</u></b></p> <ol style="list-style-type: none"> <li>1. Mixing chamber recommended but not required.</li> <li>2. Test solution completely mixed before introduction into the test system (aeration should not be used for mixing).</li> <li>3. Flow splitting accuracy must be within 10% and periodically checked.</li> </ol>	<ol style="list-style-type: none"> <li>1. The diluter system incorporated a mixing chamber.</li> <li>2. Yes</li> <li>3. The flow-splitting accuracy was &lt;4% (p. 19).</li> </ol>
<p><b><u>Exposure System/Test Vessels</u></b></p> <p>Exposure tanks should be all glass or glass with a plastic or stainless steel frame (30.5 x 30.5 x 91.4 cm or 30.5 x 30.5 x 61 cm for fathead, and 45 x 90 x 26 cm for sheepshead).</p> <p>Larval chambers should have glass bottoms and drains that allow water to be drawn down to 3 cm.</p> <p>Test water depth in adult tanks and larval chambers should be a minimum of 15 cm.</p>	<p>Glass exposure aquaria (60 x 30 x 30 cm) were used, with a fill volume of 27 L and depth of 15 cm.</p> <p>The larval growth chambers were constructed of glass and Nitex screening and measured 30 x 13 x 25 cm; two larval growth chambers were positioned within each aquarium.</p> <p>During spawning, designated aquaria each contained two spawning baskets made of 8.5-mesh nylon screening. Each basket measured 30 cm<sup>2</sup> (depth of approx. 12 cm) and was placed over a removable egg collection tray. The trays were constructed with 3-cm high glass sides and 40-mesh nylon screening bottom.</p>
<p><b><u>Embryo and Fry Chambers</u></b></p> <p>120 mL glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen. Chambers can be oscillated vertically using rocker arm apparatus (2 rpm motor) or placed in separate chambers with self-starting siphons.</p>	<p>The embryo incubation cups were 5 cm diameter glass jars with 40-mesh Nitex screen bottoms and stainless steel wire handles.</p>

Guideline Criteria	Reported Information
<b><u>Flow Rate</u></b> Flow rates to adult tanks or larval chambers should provide 90% replacement in 8-12 hours, and maintain DO at above 75% of saturation. The toxicant level cannot drop below 20% with fish in the tank.	During the pre-spawning phase, the flow rate was 7.9 volume additions per day.  During the spawning phase, the flow rate was 7.7 volume additions per day.
<b><u>Aeration</u></b> Dilution water should be aerated to insure dissolved oxygen concentrations at or near 100% saturation. Test tanks and embryo chambers should not be aerated.	Not specified

**C. Chemical System**

Guideline Criteria	Reported Information
<p><b><u>Nominal Concentrations</u></b> Minimum of 5 concentrations and a control, all replicated; plus solvent control if appropriate.</p> <p>Toxicant conc. must be measured in one tank at each toxicant level every week.</p>	<p>0 (negative control), 1.0, 2.0, 4.0, 8.0, and 16 ppb a.i.</p> <p>Toxicant concentrations were measured weekly from alternating replicate aquaria in each test group.</p>
<p><b><u>Other Variables</u></b></p> <ol style="list-style-type: none"> <li>DO must be measured at each conc. at least once a week.</li> <li>Test water temp. must be recorded continuously.</li> <li><u>Freshwater</u>: A control and one conc. must be analyzed weekly for pH, alkalinity, hardness, and conductance. <u>Natural seawater</u>: must maintain a constant salinity and not fluctuate more than 6‰ weekly; monthly pH range &lt;0.8 pH units.</li> </ol>	<ol style="list-style-type: none"> <li>DO was measured daily in each replicate aquarium.</li> <li>Temperature was measured daily in each replicate aquarium, and was also continuously monitored in one aquarium on both levels of the diluter system.</li> <li>pH and salinity were measured daily in each replicate aquarium. The salinity did not fluctuate.</li> </ol>
<p><b><u>Solvents</u></b> Should not exceed 0.1 ml/L in a flow-through system. Acceptable solvents are: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.</p>	<p>None used.</p>

**Comments:** None.

**12. REPORTED RESULTS:****A. General Results**

Guideline Criteria	Reported Information
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Guideline Criteria	Reported Information
<b>Quality assurance and GLP compliance statements were included in the report?</b>	Yes
<p><b><u>Data Endpoints must include:</u></b></p> <ul style="list-style-type: none"> <li>· survival of P and F<sub>1</sub> embryos, time required to hatch, and hatching success;</li> <li>· survival and total length of P fish at 4 and 8 weeks after hatching;</li> <li>· weights and lengths of F<sub>1</sub> fish at 8 weeks;</li> <li>· incidence of pathological or histological effects; and</li> <li>· observations of other effects or clinical signs.</li> </ul>	<p><b><u>Data Endpoints included:</u></b></p> <ul style="list-style-type: none"> <li>· survival of F<sub>0</sub> and F<sub>1</sub> embryos, time required to hatch, and hatching success;</li> <li>· survival and total lengths of F<sub>0</sub> fish at 4 and 8 weeks after hatching;</li> <li>· wet weights of fish discontinued from exposure at thinning 4 weeks after hatching;</li> <li>· survival of F<sub>0</sub> fish at 16 weeks after hatching (112 days post-hatch; test termination);</li> <li>· total lengths and weights (gender specific) of surviving F<sub>0</sub> fish at 16 weeks after hatching;</li> <li>· F<sub>0</sub> fecundity (eggs/female/day)</li> <li>· total lengths and wet weights of F<sub>1</sub> fish at 4 weeks after hatching</li> <li>· incidence of pathological or histological effects;</li> <li>· observation of other effects or clinical signs</li> </ul>
<b>Raw data included?</b>	Yes

F<sub>0</sub> Results:

Nominal Conc. (ppb a.i.)	Mean Measured Conc. (ppb a.i.) (SD)	% Hatch <sup>1</sup>	4-Week Post-Hatch % Survival	8-Week Post-Hatch % Survival <sup>2</sup>	Terminal % Survival <sup>2,3</sup>
Negative Control	<0.22	82	98	98	86
1.0	1.2 ± 0.10	86	100	100	84
2.0	2.0 ± 0.25	82	98	98	76
4.0	3.7 ± 0.28	85	99	98	80
8.0	7.4 ± 0.64	90	99	100	82
16	15 ± 1.3	77	100	98	94

Data obtained from Tables 2, p. 34, and Tables 4-6, pp. 36-38.

<sup>1</sup> Adjusted for a mean 68% viability of the eggs used to initiate the study.

<sup>2</sup> Based on 50 fish/treatment, thinned at 4 weeks post-hatch.

<sup>3</sup> 16 weeks post-hatch.

Mean Measured Conc. (ppb a.i.)	Mean Total Length (mm)					Wet Weight (g)		
	4 Weeks Post-hatch	8 Weeks Post-hatch		Test Termination		4 Weeks Post-hatch	Test Termination	
Negative Control	28.9	45.2	42.5	53.1	47.5	0.387	3.1	2.1
1.2	28.6	44.7	41.6	53.3	46.9	0.382	3.1	1.9
2.0	28.5	45.1	42.0	53.2	47.3	0.382	3.0	2.1
3.7	28.5	45.6	40.8	53.2	46.8	0.382	3.0	2.1
7.4	28.4	45.5	42.4	54.1	47.4	0.373	3.1	2.0
15	28.0*	44.4	41.9	50.9*	46.1	0.371	2.6*	1.9

Data obtained from Tables 4 and 5, pp. 36-37.

\* Significantly reduced compared to the control, based on Williams' Test.



Mean Measured Conc. (ppb a.i.)	Total Number of Eggs <sup>1</sup>	Number of Eggs/ Female/Day
Negative Control	6888	25
1.2	6622	25
2.0	6770	25
3.7	8309	30
7.4	6876	25
15	5656	22

Data obtained from Table 6, p. 38.

<sup>1</sup> Values represents mean of two replicates.

**Toxicity Observations:** A slight (3%), but statistically-significant reduction in mean total length of larval fish was observed at the 15 ppb a.i. group compared to the control (28.0 versus 28.9 mm) at 4 weeks post-hatch (Table 4, p. 36). No differences in lengths were observed at the 8-week measurement; however, at study termination, the mean total length and wet weight of males at the 15 ppb a.i. level (50.9 mm and 2.6 g) were statistically less than the controls (53.1 mm and 3.1 g). No other treatment-related effects were observed on any F<sub>0</sub> parameter. All embryo groups, at all exposure levels and the control, hatched in 6 days (p. 27). No deformities or internal abnormalities were observed among the terminated F<sub>0</sub> adult fish (p. 28).

#### F<sub>1</sub> Results:

Mean Measured Concentration (ppb a.i.)	% Hatch	4 Week Post-Hatch % Survival	4 Week Post-Hatch Length (mm)	4 Week Post-Hatch Wet Weight (g)
Negative Control	68	95	25.1	0.256
1.2	60*	95	21.1	0.160
2.0	71	98	25.0	0.251
3.7	74	93	22.6	0.173
7.4	66	100	22.5	0.175
15	52*	97	21.9	0.173

Data obtained from Table 7, p. 39.

\* Significantly reduced compared to the control, based on Fisher's Exact Test.

Mean Measured Concentration (ppb a.i.)	Percent Hatched			
	5 Days	6 Days	7 Days	8 Days
Negative Control	0	0	94	6
1.2	0	6	92	2
2.0	4	25	71	0
3.7	0	0	94	6
7.4	0	0	81	19
15	0	8	92	0

Data obtained from Table 8, p. 40.

**Toxicity Observations:** A statistically-significant reduction in hatching success was observed at the 15 ppb a.i. test group compared to the control (52 versus 68%; Table 7, p. 39). The reduced hatching success at the highest concentration was the result of the performance of one spawning group in a total of four maintained during the study (p. 28). The mean percent hatching success for the other three groups was 70%. Embryos exposed at 1.2 ppb a.i. had a statistically-significant reduction in hatching success compared to the control (60 versus 68%, respectively); however, this difference was not considered to be treatment-related as no concentration response was observed at the three higher levels. No other statistical differences were observed on any parameter assessed.

## B. Reported Statistical Results

Data obtained for the F<sub>0</sub> generation that were statistically analyzed included hatching success; 28-day post-hatch survival, total length and wet weight; 55-day post-hatch survival, male total length, and female total length; fecundity (eggs/female/day); 112-day post-hatch survival, male total length, male wet weight, female total length, and female wet weight. The time to hatch data were empirically estimated. Data obtained for the F<sub>1</sub> generation that were statistically analyzed included hatching success; time-to-hatch; and 28-day post-hatch survival, total length, and wet weight.

Continuous data (growth and reproduction endpoints) were analyzed for assumptions of normality and homogeneity, followed by William's Test using TOXSTAT (1996) statistical software. Binomial data (survival and hatching success endpoints) were analyzed by Fisher's Exact Test using SYSTAT (1999) statistical software. All statistical conclusions were made at the 95% level of certainty.

The no observed effect concentration (NOEC) is the highest test concentration causing no adverse effects. The lowest observed effect concentration (LOEC) is the lowest test concentration causing adverse effects. The maximum acceptable toxicant concentration (MATC) was calculated as the geometric mean of the NOEC and the LOEC.

Biological Endpoint	NOEC (ppb a.i.)	LOEC (ppb a.i.)	MATC (ppb a.i.)
<b>F<sub>0</sub> Generation</b>			
Hatching success	15	>15	>15
Time to hatch	15	>15	>15
4-week survival	15	>15	>15
4-week length	7.4	15	11
4-week wet weight	15	>15	>15
8-week survival	15	>15	>15
8-week length, male	15	>15	>15
8-week length, female	15	>15	>15
16-week survival	15	>15	>15
16-week length, male	7.4	15	11
16-week length, female	15	>15	>15
16-week wet weight, male	7.4	15	11
16-week wet weight, female	15	>15	>15
Fecundity (eggs/female/day)	15	>15	>15
<b>F<sub>1</sub> Generation</b>			
Hatching success	7.4	15	11
Time to hatch	15	>15	>15
4-week survival	15	>15	>15
4-week length	15	>15	>15

Biological Endpoint	NOEC (ppb a.i.)	LOEC (ppb a.i.)	MATC (ppb a.i.)
4-week weight	15	>15	>15
8-week survival	Not determined	Not determined	Not determined
8-week length	Not determined	Not determined	Not determined
8-week weight	Not determined	Not determined	Not determined

**NOEC:** 7.4 ppb a.i.

**LOEC:** 15 ppb a.i.

**MATC:** 11 ppb a.i.

**Endpoint(s) Affected:** F<sub>0</sub> 4-week post-hatch length; F<sub>0</sub> 16-week (terminal) post-hatch length and wet weight of males; and F<sub>1</sub> hatching success (same conclusions)

### 13. REVIEWER'S STATISTICAL RESULTS:

Data obtained for the F<sub>0</sub> generation that were statistically analyzed included hatching success, 28-day post-hatch survival, total length and wet weight, 55-day post-hatch survival, male total length, and female total length, fecundity (eggs/female/day), 112-day post-hatch survival, male total length, male wet weight, female total length, and female wet weight. The time to hatch data were also statistically analyzed by comparing the number of groups hatching following treatment on days 6, 7, and 8. Data obtained for the F<sub>1</sub> generation that were statistically analyzed included hatching success, 28-day post-hatch survival, total length, and wet weight.

All data were analyzed to determine if they satisfied the assumptions of ANOVA (i.e., normality and homogeneity of variances). If they did, the NOEC and LOEC were determined using this test via TOXSTAT statistical software. If data did not satisfy these assumptions, the NOEC and LOEC values were determined using the non-parametric Kruskal-Wallis test. These analyses were conducted using TOXSTAT statistical software.

Biological Endpoint	NOEC (ppb a.i.)	LOEC (ppb a.i.)
<b>F<sub>0</sub> Generation</b>		
Hatching success	15	>15
Time to hatch	15	>15
4-week survival	15	>15
4-week length	15	>15
4-week wet weight	15	>15
8-week survival	15	>15
8-week length, male	15	>15
8-week length, female	15	>15
16-week survival	15	>15
16-week length, male	15	>15
16-week length, female	15	>15
16-week wet weight, male	15	>15
16-week wet weight, female	15	>15
Fecundity (eggs/female/day)	15	>15
<b>F<sub>1</sub> Generation</b>		
Hatching success	15	>15
Time to hatch	15	>15
4-week survival	15	>15
4-week length	15	>15
4-week weight	15	>15

The reviewer's analyses revealed no significant effects of treatment on any parameter.

#### 14. REVIEWER'S COMMENTS:

Quality control samples were prepared at each sampling interval and remained with the set of exposure solution samples throughout the analytical process; samples were prepared in saltwater at a nominal concentrations of 0.500, 4.00, and 20.0 ppb a.i. (p. 24 and Table 3, p. 35). Recoveries ranged from 82.6-128% (n=51, includes two outliers >120%).

A method validation study conducted prior to initiation of the definitive test established a mean recovery of  $91.8 \pm 7.47\%$  for ethoprop from filtered seawater (Table 1B, p. 91). The LOQ was 0.136 ppb a.i.

This study was performed according to U.S. EPA (FIFRA) Good Laboratory Practice Standards (40 CFR 160, 1993) with the exception of the collection for the water and food contaminant screening analyses. A Quality Assurance Statement was provided.

The reviewer's conclusions differed from the study author's, in that the reviewer's analyses revealed no significant effects of treatment on any endpoint, while the study author's analyses revealed significant reductions in  $F_0$  4-week post-hatch length,  $F_0$  16-week (terminal) post-hatch length and wet weight of males, and  $F_1$  hatching success. Differences in these conclusions are attributed to the different statistical methods that these results are based on. In the reviewer's analysis, ANOVA did not reveal any significant differences among the control and treatment groups, so *post-hoc* multiple comparison tests (i.e., William's test) were not warranted. However, there were a number of significant deviations from recommended protocol that resulted in an **INVALID** classification of the study by the reviewer. In particular, an inappropriate number of replicates are used and the ability of this study to detect significant treatment effects is compromised. In addition, guideline requirements specify that the hatching success of the controls for sheepshead minnows should be >75%; however, the mean hatching success of the controls in this study (57%; page 27 of the study report) does not meet guideline requirements. Subsequently, the ability of this study to detect treatment-related effects on this endpoint may be compromised and it is highly uncertain how overall poor hatching success of the source population for test subjects may influence the estimation of other endpoints. This study is not upgradable and does fulfill the guideline requirement for a full life-cycle toxicity test (§72-5). Therefore, EFED recommends that a new full life-cycle study establishing a reliable NOAEC for estuarine/marine fish be conducted.

**15. REFERENCES:**

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DP Barcode: D306508

MRID No: 46315401

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**APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:**

hatching success

File: 5401hs Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	80.667	16.133	1.152
Within (Error)	6	84.000	14.000	
Total	11	164.667		

Critical F value = 4.39 (0.05,5,6)

Since  $F < \text{Critical } F$  FAIL TO REJECT  $H_0$ : All groups equal

hatching success

File: 5401hs Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2  $H_0$ : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	56.500	56.500		
2	1.2	59.000	59.000	-0.668	
3	2.0	56.500	56.500	0.000	
4	3.7	57.500	57.500	-0.267	
5	7.4	61.500	61.500	-1.336	
6	15	53.000	53.000	0.935	

Dunnett table value = 2.83 (1 Tailed Value,  $P=0.05$ ,  $df=6,5$ )

hatching success

File: 5401hs Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2  $H_0$ : Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of DIFFERENCE CONTROL FROM CONTROL
1	control	2		
2	1.2	2	10.589	18.7 -2.500
3	2.0	2	10.589	18.7 0.000
4	3.7	2	10.589	18.7 -1.000
5	7.4	2	10.589	18.7 -5.000

DP Barcode: D306508

MRID No: 46315401

6 15 2 10.589 18.7 3.500

hatching success

File: 5401hs Transform: NO TRANSFORMATION

WILLIAMS TEST (isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	56.500	56.500	58.200
2	1.2	2	59.000	59.000	58.200
3	2.0	2	56.500	56.500	58.200
4	3.7	2	57.500	57.500	58.200
5	7.4	2	61.500	61.500	58.200
6	15	2	53.000	53.000	53.000

hatching success

File: 5401hs Transform: NO TRANSFORMATION

WILLIAMS TEST (isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. SIG WILLIAMS	TABLE P=.05	DEGREES OF WILLIAMS FREEDOM
control	58.200			
1.2	58.200	0.454	1.94	k= 1, v= 6
2.0	58.200	0.454	2.06	k= 2, v= 6
3.7	58.200	0.454	2.10	k= 3, v= 6
7.4	58.200	0.454	2.12	k= 4, v= 6
15	53.000	0.935	2.13	k= 5, v= 6

s = 3.742

Note: df used for table values are approximate when v > 20.

hatching success (% viability)

File: 5401hsv Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	194.667	38.933	1.557
Within (Error)	6	150.000	25.000	
Total	11	344.667		

Critical F value = 4.39 (0.05,5,6)

Since  $F < \text{Critical } F$  FAIL TO REJECT  $H_0$ : All groups equal

hatching success (% viability)

File: 5401hsv Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2

$H_0$ : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	81.500	81.500		
2	1.2	85.500	85.500	-0.800	
3	2.0	81.500	81.500	0.000	
4	3.7	84.500	84.500	-0.600	
5	7.4	90.000	90.000	-1.700	
6	15	77.000	77.000	0.900	

Dunnett table value = 2.83 (1 Tailed Value,  $P=0.05$ ,  $df=6,5$ )

hatching success (% viability)

File: 5401hsv Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2

$H_0$ : Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of DIFFERENCE CONTROL FROM CONTROL
1	control	2		
2	1.2	2	14.150	17.4 -4.000
3	2.0	2	14.150	17.4 0.000
4	3.7	2	14.150	17.4 -3.000
5	7.4	2	14.150	17.4 -8.500
6	15	2	14.150	17.4 4.500

hatching success (% viability)

File: 5401hsv Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	81.500	81.500	84.600
2	1.2	2	85.500	85.500	84.600

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3	2.0	2	81.500	81.500	84.600
4	3.7	2	84.500	84.500	84.600
5	7.4	2	90.000	90.000	84.600
6	15	2	77.000	77.000	77.000

hatching success (% viability)

File: 5401hsv Transform: NO TRANSFORMATION

WILLIAMS TEST (isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE DEGREES OF WILLIAMS	FREEDOM
control	84.600				
1.2	84.600	0.620	1.94	k= 1, v= 6	
2.0	84.600	0.620	2.06	k= 2, v= 6	
3.7	84.600	0.620	2.10	k= 3, v= 6	
7.4	84.600	0.620	2.12	k= 4, v= 6	
15	77.000	0.900	2.13	k= 5, v= 6	

s = 5.000

Note: df used for table values are approximate when v > 20.

day 28 length

File: 5401I28 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.998	0.200	2.198
Within (Error)	6	0.545	0.091	
Total	11	1.543		

Critical F value = 4.39 (0.05,5,6)

Since F < Critical F FAIL TO REJECT Ho:All groups equal

day 28 length

File: 5401I28 Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	28.950	28.950		

DP Barcode: D306508

MRID No: 46315401

2	1.2	28.650	28.650	0.994
3	2.0	28.450	28.450	1.657
4	3.7	28.450	28.450	1.657
5	7.4	28.350	28.350	1.989
6	15	28.000	28.000	3.149 *

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

day 28 length

File: 5401I28 Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of DIFFERENCE CONTROL FROM CONTROL
1	control	2		
2	1.2	2	0.854	2.9 0.300
3	2.0	2	0.854	2.9 0.500
4	3.7	2	0.854	2.9 0.500
5	7.4	2	0.854	2.9 0.600
6	15	2	0.854	2.9 0.950

day 28 length

File: 5401I28 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	28.950	28.950	28.950
2	1.2	2	28.650	28.650	28.650
3	2.0	2	28.450	28.450	28.450
4	3.7	2	28.450	28.450	28.450
5	7.4	2	28.350	28.350	28.350
6	15	2	28.000	28.000	28.000

day 28 length

File: 5401I28 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
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control	28.950				
1.2	28.650	0.995	1.94	k= 1, v= 6	
2.0	28.450	1.659	2.06	k= 2, v= 6	
3.7	28.450	1.659	2.10	k= 3, v= 6	
7.4	28.350	1.991	2.12	k= 4, v= 6	
15	28.000	3.152	* 2.13	k= 5, v= 6	

s = 0.301

Note: df used for table values are approximate when v &gt; 20.

day 28 weight

File: 5401w28 Transform: NO TRANSFORMATION

## ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	387.000	77.400	0.308
Within (Error)	6	1510.000	251.667	
Total	11	1897.000		

Critical F value = 4.39 (0.05,5,6)

Since F &lt; Critical F FAIL TO REJECT Ho:All groups equal

day 28 weight

File: 5401w28 Transform: NO TRANSFORMATION

## DUNNETTS TEST - TABLE 1 OF 2 Ho:Control&lt;Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	387.500	387.500		
2	1.2	381.500	381.500	0.378	
3	2.0	382.000	382.000	0.347	
4	3.7	382.000	382.000	0.347	
5	7.4	372.500	372.500	0.946	
6	15	371.500	371.500	1.009	

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

day 28 weight

File: 5401w28 Transform: NO TRANSFORMATION

## DUNNETTS TEST - TABLE 2 OF 2 Ho:Control&lt;Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of DIFFERENCE CONTROL FROM CONTROL
1	control	2		
2	1.2	2	44.895	11.6
3	2.0	2	44.895	11.6
4	3.7	2	44.895	11.6
5	7.4	2	44.895	11.6
6	15	2	44.895	11.6

day 28 weight

File: 5401w28 Transform: NO TRANSFORMATION

WILLIAMS TEST (isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	387.500	387.500	387.500
2	1.2	2	381.500	381.500	381.833
3	2.0	2	382.000	382.000	381.833
4	3.7	2	382.000	382.000	381.833
5	7.4	2	372.500	372.500	372.500
6	15	2	371.500	371.500	371.500

day 28 weight

File: 5401w28 Transform: NO TRANSFORMATION

WILLIAMS TEST (isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. SIG WILLIAMS P=.05	TABLE DEGREES OF FREEDOM
control	387.500		
1.2	381.833	0.357	1.94 k= 1, v= 6
2.0	381.833	0.357	2.06 k= 2, v= 6
3.7	381.833	0.357	2.10 k= 3, v= 6
7.4	372.500	0.946	2.12 k= 4, v= 6
15	371.500	1.009	2.13 k= 5, v= 6

s = 15.864

Note: df used for table values are approximate when v &gt; 20.

day 55 length males

File: 5401i55 Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	45.250	45.250	13.000
2	1.2	44.750	44.750	10.500
3	2.0	45.100	45.100	9.500
4	3.7	45.600	45.600	18.500
5	7.4	45.500	45.500	21.000
6	15	44.450	44.450	5.500

Calculated H Value = 6.592      Critical H Value Table = 11.070  
 Since Calc H < Crit H FAIL TO REJECT Ho: All groups are equal.

day 55 length males

File: 5401I55      Transform: NO TRANSFORM

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	0 0 0 0 0	6 2 3 1 5 4
6	15	44.450	44.450 \		
2	1.2	44.750	44.750 . \		
3	2.0	45.100	45.100 .. \		
1	control	45.250	45.250 ... \		
5	7.4	45.500	45.500 .... \		
4	3.7	45.600	45.600 ..... \		

\* = significant difference (p=0.05)      . = no significant difference  
 Table q value (0.05,6) = 2.936      SE = 3.580

day 55 female length

File: 540155f      Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	4.537	0.907	0.696
Within (Error)	6	7.820	1.303	
Total	11	12.357		

Critical F value = 4.39 (0.05,5,6)  
 Since F < Critical F FAIL TO REJECT Ho: All groups equal



day 55 female length

File: 540155f Transform: NO TRANSFORMATION

## DUNNETTS TEST - TABLE 1 OF 2

Ho:Control&lt;Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	42.550	42.550		
2	1.2	41.600	41.600	0.832	
3	2.0	42.000	42.000	0.482	
4	3.7	40.750	40.750	1.577	
5	7.4	42.550	42.550	0.000	
6	15	41.850	41.850	0.613	

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

day 55 female length

File: 540155f Transform: NO TRANSFORMATION

## DUNNETTS TEST - TABLE 2 OF 2

Ho:Control&lt;Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of DIFFERENCE CONTROL FROM CONTROL
1	control	2		
2	1.2	2	3.230	7.6
3	2.0	2	3.230	7.6
4	3.7	2	3.230	7.6
5	7.4	2	3.230	7.6
6	15	2	3.230	7.6

day 55 female length

File: 540155f Transform: NO TRANSFORMATION

## WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	42.550	42.550	42.550
2	1.2	2	41.600	41.600	41.800
3	2.0	2	42.000	42.000	41.800
4	3.7	2	40.750	40.750	41.717
5	7.4	2	42.550	42.550	41.717
6	15	2	41.850	41.850	41.717

day 55 female length

File: 540155f Transform: NO TRANSFORMATION

## WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE DEGREES OF WILLIAMS FREEDOM
control	42.550			
1.2	41.800	0.657	1.94	k= 1, v= 6
2.0	41.800	0.657	2.06	k= 2, v= 6
3.7	41.717	0.730	2.10	k= 3, v= 6
7.4	41.717	0.730	2.12	k= 4, v= 6
15	41.717	0.730	2.13	k= 5, v= 6

s = 1.142

Note: df used for table values are approximate when v &gt; 20.

F0 survival

File: 5401s Transform: NO TRANSFORMATION

## ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	374.667	74.933	0.301
Within (Error)	6	1496.000	249.333	
Total	11	1870.667		

Critical F value = 4.39 (0.05,5,6)

Since F &lt; Critical F FAIL TO REJECT Ho:All groups equal

F0 survival

File: 5401s Transform: NO TRANSFORMATION

## DUNNETTS TEST - TABLE 1 OF 2

Ho:Control&lt;Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	86.000	86.000		
2	1.2	84.000	84.000	0.127	
3	2.0	76.000	76.000	0.633	
4	3.7	80.000	80.000	0.380	
5	7.4	82.000	82.000	0.253	

DP Barcode: D306508

MRID No: 46315401

6 15 94.000 94.000 -0.507

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

F0 survival

File: 5401s Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of DIFFERENCE CONTROL FROM CONTROL
1	control	2		
2	1.2	2	44.686	52.0
3	2.0	2	44.686	52.0
4	3.7	2	44.686	52.0
5	7.4	2	44.686	52.0
6	15	2	44.686	52.0

F0 survival

File: 5401s Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	86.000	86.000	81.500
2	1.2	2	84.000	84.000	81.500
3	2.0	2	76.000	76.000	81.500
4	3.7	2	80.000	80.000	81.500
5	7.4	2	82.000	82.000	82.000
6	15	2	94.000	94.000	94.000

F0 survival

File: 5401s Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=05	TABLE DEGREES OF FREEDOM
control	81.500			
1.2	81.500	0.285	1.94	k= 1, v= 6
2.0	81.500	0.285	2.06	k= 2, v= 6
3.7	81.500	0.285	2.10	k= 3, v= 6

DP Barcode: D306508

MRID No: 46315401

7.4	82.000	0.253	2.12	k= 4, v= 6
15	94.000	0.507	2.13	k= 5, v= 6

s = 15.790

Note: df used for table values are approximate when v > 20.

F0 male length

File: 5401ml Transform: NO TRANSFORMATION

#### ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	12.867	2.573	3.034
Within (Error)	6	5.090	0.848	
Total	11	17.957		

Critical F value = 4.39 (0.05,5,6)

Since F < Critical F FAIL TO REJECT Ho:All groups equal

F0 male length

File: 5401ml Transform: NO TRANSFORMATION

#### DUNNETTS TEST - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	53.000	53.000		
2	1.2	53.350	53.350	-0.380	
3	2.0	53.300	53.300	-0.326	
4	3.7	53.400	53.400	-0.434	
5	7.4	54.200	54.200	-1.303	
6	15	50.850	50.850	2.335	

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

F0 male length

File: 5401ml Transform: NO TRANSFORMATION

#### DUNNETTS TEST - TABLE 2 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of DIFFERENCE CONTROL FROM CONTROL
1	control	2		

DP Barcode: D306508

MRID No: 46315401

2	1.2	2	2.606	4.9	-0.350
3	2.0	2	2.606	4.9	-0.300
4	3.7	2	2.606	4.9	-0.400
5	7.4	2	2.606	4.9	-1.200
6	15	2	2.606	4.9	2.150

F0 male length

File: 5401ml Transform: NO TRANSFORMATION

WILLIAMS TEST (isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	53.000	53.000	53.450
2	1.2	2	53.350	53.350	53.450
3	2.0	2	53.300	53.300	53.450
4	3.7	2	53.400	53.400	53.450
5	7.4	2	54.200	54.200	53.450
6	15	2	50.850	50.850	50.850

F0 male length

File: 5401ml Transform: NO TRANSFORMATION

WILLIAMS TEST (isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. SIG WILLIAMS	TABLE P=.05	DEGREES OF WILLIAMS	FREEDOM
control	53.450				
1.2	53.450	0.489	1.94	k= 1, v= 6	
2.0	53.450	0.489	2.06	k= 2, v= 6	
3.7	53.450	0.489	2.10	k= 3, v= 6	
7.4	53.450	0.489	2.12	k= 4, v= 6	
15	50.850	2.334 *	2.13	k= 5, v= 6	

s = 0.921

Note: df used for table values are approximate when v > 20.

F0 female length

File: 5401fl Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	2.907	0.581	0.236

Within (Error)	6	14.760	2.460
Total	11	17.667	

Critical F value = 4.39 (0.05,5,6)

Since  $F < \text{Critical } F$  FAIL TO REJECT  $H_0$ : All groups equal

F0 female length

File: 5401fl Transform: NO TRANSFORMATION

## DUNNETTS TEST - TABLE 1 OF 2

 $H_0$ : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	47.400	47.400		
2	1.2	47.000	47.000	0.255	
3	2.0	47.100	47.100	0.191	
4	3.7	46.300	46.300	0.701	
5	7.4	47.300	47.300	0.064	
6	15	46.100	46.100	0.829	

Dunnett table value = 2.83 (1 Tailed Value,  $P=0.05$ ,  $df=6,5$ )

F0 female length

File: 5401fl Transform: NO TRANSFORMATION

## DUNNETTS TEST - TABLE 2 OF 2

 $H_0$ : Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of DIFFERENCE CONTROL FROM CONTROL
1	control	2		
2	1.2	2	4.439	9.4
3	2.0	2	4.439	9.4
4	3.7	2	4.439	9.4
5	7.4	2	4.439	9.4
6	15	2	4.439	9.4

F0 female length

File: 5401fl Transform: NO TRANSFORMATION

## WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	ORIGINAL	TRANSFORMED	ISOTONIZED
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	IDENTIFICATION	N	MEAN	MEAN	MEAN
1	control	2	47.400	47.400	47.400
2	1.2	2	47.000	47.000	47.050
3	2.0	2	47.100	47.100	47.050
4	3.7	2	46.300	46.300	46.800
5	7.4	2	47.300	47.300	46.800
6	15	2	46.100	46.100	46.100

F0 female length

File: 5401fl Transform: NO TRANSFORMATION

## WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. SIG WILLIAMS	TABLE P=.05	DEGREES OF WILLIAMS	FREEDOM
control	47.400				
1.2	47.050	0.223	1.94	k= 1, v= 6	
2.0	47.050	0.223	2.06	k= 2, v= 6	
3.7	46.800	0.383	2.10	k= 3, v= 6	
7.4	46.800	0.383	2.12	k= 4, v= 6	
15	46.100	0.829	2.13	k= 5, v= 6	

s = 1.568

Note: df used for table values are approximate when v &gt; 20.

F0 male weight

File: 5401mw Transform: NO TRANSFORMATION

## ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.467	0.093	2.906
Within (Error)	6	0.190	0.032	
Total	11	0.657		

Critical F value = 4.39 (0.05,5,6)

Since F &lt; Critical F FAIL TO REJECT Ho: All groups equal

F0 male weight

File: 5401mw Transform: NO TRANSFORMATION

## DUNNETTS TEST - TABLE 1 OF 2

Ho: Control &lt; Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	3.050	3.050		
2	1.2	3.050	3.050	0.000	
3	2.0	3.050	3.050	0.000	
4	3.7	3.050	3.050	0.000	
5	7.4	3.150	3.150	-0.559	
6	15	2.550	2.550	2.795	

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

F0 male weight

File: 5401mw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	2			
2	1.2	2	0.506	16.6	0.000
3	2.0	2	0.506	16.6	0.000
4	3.7	2	0.506	16.6	0.000
5	7.4	2	0.506	16.6	-0.100
6	15	2	0.506	16.6	0.500

F0 male weight

File: 5401mw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	3.050	3.050	3.070
2	1.2	2	3.050	3.050	3.070
3	2.0	2	3.050	3.050	3.070
4	3.7	2	3.050	3.050	3.070
5	7.4	2	3.150	3.150	3.070
6	15	2	2.550	2.550	2.550

F0 male weight

File: 5401mw Transform: NO TRANSFORMATION



## WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG	TABLE P=.05	DEGREES OF WILLIAMS	FREEDOM
control	3.070					
1.2	3.070	0.112	1.94	k= 1, v= 6		
2.0	3.070	0.112	2.06	k= 2, v= 6		
3.7	3.070	0.112	2.10	k= 3, v= 6		
7.4	3.070	0.112	2.12	k= 4, v= 6		
15	2.550	2.810	*	2.13	k= 5, v= 6	

s = 0.178

Note: df used for table values are approximate when v &gt; 20.

F0 female weight

File: 5401fw Transform: NO TRANSFORM

## KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	2.100	2.100	17.500
2	1.2	2.000	2.000	13.000
3	2.0	2.050	2.050	14.500
4	3.7	2.000	2.000	13.000
5	7.4	2.000	2.000	14.000
6	15	1.850	1.850	6.000

Calculated H Value = 2.879 Critical H Value Table = 11.070

Since Calc H &lt; Crit H FAIL TO REJECT Ho: All groups are equal.

F0 female weight

File: 5401fw Transform: NO TRANSFORM

## DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	GROUP ORIGINAL MEAN	0 0 0 0 0	6 4 5 2 3 1
6	15	1.850	1.850 \		
4	3.7	2.000	2.000 . \		
5	7.4	2.000	2.000 .. \		
2	1.2	2.000	2.000 ... \		
3	2.0	2.050	2.050 .... \		
1	control	2.100	2.100 ..... \		

\* = significant difference (p=0.05)

. = no significant difference

DP Barcode: D306508

MRID No: 46315401

Table q value (0.05,6) = 2.936 SE = 3.548

total eggs

File: 5401e Transform: NO TRANSFORMATION

# ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1807373.750	361474.750	0.874
Within (Error)	6	2482450.500	413741.750	
Total	11	4289824.250		

Critical F value = 4.39 (0.05,5,6)

Since F < Critical F FAIL TO REJECT Ho:All groups equal

total eggs

File: 5401e Transform: NO TRANSFORMATION

# DUNNETTS TEST - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	3444.000	3444.000		
2	1.2	3311.000	3311.000	0.207	
3	2.0	3385.000	3385.000	0.092	
4	3.7	4154.500	4154.500	-1.105	
5	7.4	3438.000	3438.000	0.009	
6	15	2828.000	2828.000	0.958	

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

total eggs

File: 5401e Transform: NO TRANSFORMATION

# DUNNETTS TEST - TABLE 2 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of DIFFERENCE CONTROL FROM CONTROL
1	control	2		
2	1.2	2	1820.334	52.9 133.000
3	2.0	2	1820.334	52.9 59.000
4	3.7	2	1820.334	52.9 -710.500
5	7.4	2	1820.334	52.9 6.000

DP Barcode: D306508

MRID No: 46315401

6 15 2 1820.334 52.9 616.000

total eggs

File: 5401e Transform: NO TRANSFORMATION

WILLIAMS TEST (isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	3444.000	3444.000	3573.625
2	1.2	2	3311.000	3311.000	3573.625
3	2.0	2	3385.000	3385.000	3573.625
4	3.7	2	4154.500	4154.500	3573.625
5	7.4	2	3438.000	3438.000	3438.000
6	15	2	2828.000	2828.000	2828.000

total eggs

File: 5401e Transform: NO TRANSFORMATION

WILLIAMS TEST (isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED CALC. MEAN	SIG WILLIAMS	TABLE P=.05	DEGREES OF WILLIAMS	FREEDOM
control	3573.625				
1.2	3573.625	0.202	1.94	k= 1, v= 6	
2.0	3573.625	0.202	2.06	k= 2, v= 6	
3.7	3573.625	0.202	2.10	k= 3, v= 6	
7.4	3438.000	0.009	2.12	k= 4, v= 6	
15	2828.000	0.958	2.13	k= 5, v= 6	

s = 643.228

Note: df used for table values are approximate when v > 20.

eggs/female/day

File: 5401ed Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	74.750	14.950	0.790
Within (Error)	6	113.500	18.917	
Total	11	188.250		

Critical F value = 4.39 (0.05,5,6)

Since  $F < \text{Critical } F$  FAIL TO REJECT  $H_0$ : All groups equal

eggs/female/day

File: 5401ed Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2  $H_0$ : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	25.500	25.500		
2	1.2	24.500	24.500	0.230	
3	2.0	25.000	25.000	0.115	
4	3.7	30.000	30.000	-1.035	
5	7.4	25.000	25.000	0.115	
6	15	21.500	21.500	0.920	

Dunnett table value = 2.83 (1 Tailed Value,  $P=0.05$ ,  $df=6,5$ )

eggs/female/day

File: 5401ed Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2  $H_0$ : Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of DIFFERENCE CONTROL FROM CONTROL
1	control	2		
2	1.2	2	12.309	48.3
3	2.0	2	12.309	48.3
4	3.7	2	12.309	48.3
5	7.4	2	12.309	48.3
6	15	2	12.309	48.3

eggs/female/day

File: 5401ed Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	25.500	25.500	26.250
2	1.2	2	24.500	24.500	26.250

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3	2.0	2	25.000	25.000	26.250
4	3.7	2	30.000	30.000	26.250
5	7.4	2	25.000	25.000	25.000
6	15	2	21.500	21.500	21.500

eggs/female/day

File: 5401ed Transform: NO TRANSFORMATION

WILLIAMS TEST (isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	MEAN	ISOTONIZED CALC.	SIG WILLIAMS	TABLE P=.05	DEGREES OF WILLIAMS	FREEDOM
control	26.250					
1.2	26.250	0.172	1.94	k= 1, v= 6		
2.0	26.250	0.172	2.06	k= 2, v= 6		
3.7	26.250	0.172	2.10	k= 3, v= 6		
7.4	25.000	0.115	2.12	k= 4, v= 6		
15	21.500	0.920	2.13	k= 5, v= 6		

s = 4.349

Note: df used for table values are approximate when v > 20.

hatching success F1

File: 5401h1 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	616.417	123.283	1.702
Within (Error)	6	434.500	72.417	
Total	11	1050.917		

Critical F value = 4.39 (0.05,5,6)

Since F < Critical F FAIL TO REJECT Ho:All groups equal

hatching success F1

File: 5401h1 Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
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1	control	68.000	68.000	
2	1.2	59.000	59.000	1.058
3	2.0	70.000	70.000	-0.235
4	3.7	74.000	74.000	-0.705
5	7.4	66.000	66.000	0.235
6	15	52.500	52.500	1.821

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

hatching success F1

File: 5401h1 Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of DIFFERENCE CONTROL FROM CONTROL
1	control	2		
2	1.2	2	24.083	35.4 9.000
3	2.0	2	24.083	35.4 -2.000
4	3.7	2	24.083	35.4 -6.000
5	7.4	2	24.083	35.4 2.000
6	15	2	24.083	35.4 15.500

hatching success F1

File: 5401h1 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	68.000	68.000	68.000
2	1.2	2	59.000	59.000	67.667
3	2.0	2	70.000	70.000	67.667
4	3.7	2	74.000	74.000	67.667
5	7.4	2	66.000	66.000	66.000
6	15	2	52.500	52.500	52.500

hatching success F1

File: 5401h1 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED CALC. MEAN	SIG WILLIAMS P=.05	TABLE DEGREES OF WILLIAMS FREEDOM
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control	68.000			
1.2	67.667	0.039	1.94	k= 1, v= 6
2.0	67.667	0.039	2.06	k= 2, v= 6
3.7	67.667	0.039	2.10	k= 3, v= 6
7.4	66.000	0.235	2.12	k= 4, v= 6
15	52.500	1.821	2.13	k= 5, v= 6

s = 8.510

Note: df used for table values are approximate when v &gt; 20.

survival F1

File: 5401s1 Transform: NO TRANSFORM

## KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	95.000	95.000	12.000
2	1.2	95.000	95.000	10.500
3	2.0	98.000	98.000	15.500
4	3.7	93.000	93.000	6.500
5	7.4	100.000	100.000	21.000
6	15	97.000	97.000	12.500

Calculated H Value = 4.889 Critical H Value Table = 11.070  
 Since Calc H < Crit H FAIL TO REJECT Ho: All groups are equal.

survival F1

File: 5401s1 Transform: NO TRANSFORM

## DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	GROUP ORIGINAL MEAN	0 0 0 0 0	4 1 2 6 3 5
4	3.7	93.000	93.000 \		
1	control	95.000	95.000 . \		
2	1.2	95.000	95.000 .. \		
6	15	97.000	97.000 ... \		
3	2.0	98.000	98.000 .... \		
5	7.4	100.000	100.000 ..... \		

\* = significant difference (p=0.05) . = no significant difference  
 Table q value (0.05,6) = 2.936 SE = 3.503

length F1

File: 5401l1 Transform: NO TRANSFORMATION

## ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	27.064	5.413	0.935
Within (Error)	6	34.725	5.787	
Total	11	61.789		

Critical F value = 4.39 (0.05,5,6)

Since  $F < \text{Critical } F$  FAIL TO REJECT  $H_0$ : All groups equal

length F1

File: 540111 Transform: NO TRANSFORMATION

## DUNNETTS TEST - TABLE 1 OF 2

 $H_0$ : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	25.050	25.050		
2	1.2	21.000	21.000	1.684	
3	2.0	24.950	24.950	0.042	
4	3.7	22.650	22.650	0.998	
5	7.4	22.450	22.450	1.081	
6	15	21.950	21.950	1.289	

Dunnett table value = 2.83 (1 Tailed Value,  $P=0.05$ ,  $df=6,5$ )

length F1

File: 540111 Transform: NO TRANSFORMATION

## DUNNETTS TEST - TABLE 2 OF 2

 $H_0$ : Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of DIFFERENCE CONTROL FROM CONTROL
1	control	2		
2	1.2	2	6.808	27.2
3	2.0	2	6.808	27.2
4	3.7	2	6.808	27.2
5	7.4	2	6.808	27.2
6	15	2	6.808	27.2



DP Barcode: D306508

MRID No: 46315401

length F1

File: 540111 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	25.050	25.050	25.050
2	1.2	2	21.000	21.000	22.975
3	2.0	2	24.950	24.950	22.975
4	3.7	2	22.650	22.650	22.650
5	7.4	2	22.450	22.450	22.450
6	15	2	21.950	21.950	21.950

length F1

File: 540111 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. SIG WILLIAMS	TABLE P=.05	DEGREES OF WILLIAMS	FREEDOM
control	25.050				
1.2	22.975	0.863	1.94	k= 1, v= 6	
2.0	22.975	0.863	2.06	k= 2, v= 6	
3.7	22.650	0.998	2.10	k= 3, v= 6	
7.4	22.450	1.081	2.12	k= 4, v= 6	
15	21.950	1.289	2.13	k= 5, v= 6	

s = 2.406

Note: df used for table values are approximate when v > 20.

weight F1

File: 5401w1 Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	256.000	256.000	21.000
2	1.2	157.150	157.150	9.500
3	2.0	251.000	251.000	20.500
4	3.7	175.000	175.000	8.500
5	7.4	175.500	175.500	9.500
6	15	173.000	173.000	9.000

Calculated H Value = 7.035

Critical H Value Table = 11.070

Since  $\text{Calc } H < \text{Crit } H$  FAIL TO REJECT  $H_0$ : All groups are equal.

weight F1

File: 5401w1 Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

		GROUP			
		TRANSFORMED	ORIGINAL	0 0 0 0 0	
GROUP	IDENTIFICATION	MEAN	MEAN	2 6 4 5 3 1	
2	1.2	157.150	157.150 \		
6	15	173.000	173.000 . \		
4	3.7	175.000	175.000 .. \		
5	7.4	175.500	175.500 ... \		
3	2.0	251.000	251.000 .... \		
1	control	256.000	256.000 ..... \		

\* = significant difference ( $p=0.05$ )

. = no significant difference

Table q value (0.05,6) = 2.936

SE = 3.587

%hatch 6 days

File: 5401h6 Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

		TRANSFORMED		MEAN CALCULATED IN		RANK SUM
GROUP	IDENTIFICATION	MEAN	ORIGINAL	UNITS		
1	control	0.000	0.000	9.000		
2	1.2	5.500	5.500	19.000		
3	2.0	25.000	25.000	16.500		
4	3.7	0.000	0.000	9.000		
5	7.4	0.000	0.000	9.000		
6	15	7.500	7.500	15.500		

Calculated H Value = 5.582 Critical H Value Table = 11.070

Since  $\text{Calc } H < \text{Crit } H$  FAIL TO REJECT  $H_0$ : All groups are equal.

%hatch 6 days

File: 5401h6 Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

		GROUP			
		TRANSFORMED	ORIGINAL	0 0 0 0 0	
GROUP	IDENTIFICATION	MEAN	MEAN	1 4 5 2 6 3	

DP Barcode: D306508

MRID No: 46315401

1	control	0.000	0.000 \
4	3.7	0.000	0.000 . \
5	7.4	0.000	0.000 .. \
2	1.2	5.500	5.500 ... \
6	15	7.500	7.500 .... \
3	2.0	25.000	25.000 ..... \

\* = significant difference (p=0.05)      . = no significant difference  
 Table q value (0.05,6) = 2.936      SE = 3.030

#hatch 7 days

File: 5401h7      Transform: NO TRANSFORMATION

#### ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	889.417	177.883	0.407
Within (Error)	6	2619.500	436.583	
Total	11	3508.917		

Critical F value = 4.39 (0.05,5,6)

Since F < Critical F FAIL TO REJECT Ho:All groups equal

#hatch 7 days

File: 5401h7      Transform: NO TRANSFORMATION

#### DUNNETTS TEST - TABLE 1 OF 2      Ho:Control<Treatment

GROUP	IDENTIFICATION	MEAN	TRANSFORMED MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	94.500	94.500		
2	1.2	92.000	92.000	0.120	
3	2.0	71.000	71.000	1.125	
4	3.7	94.000	94.000	0.024	
5	7.4	81.500	81.500	0.622	
6	15	92.500	92.500	0.096	

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

#hatch 7 days

File: 5401h7      Transform: NO TRANSFORMATION

#### DUNNETTS TEST - TABLE 2 OF 2      Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of DIFFERENCE CONTROL FROM CONTROL
1	control	2		
2	1.2	2	59.132	62.6
3	2.0	2	59.132	62.6
4	3.7	2	59.132	62.6
5	7.4	2	59.132	62.6
6	15	2	59.132	62.6

#hatch 7 days

File: 5401h7 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	94.500	94.500	94.500
2	1.2	2	92.000	92.000	92.000
3	2.0	2	71.000	71.000	84.750
4	3.7	2	94.000	94.000	84.750
5	7.4	2	81.500	81.500	84.750
6	15	2	92.500	92.500	84.750

#hatch 7 days

File: 5401h7 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P $\approx$ .05	TABLE DEGREES OF WILLIAMS FREEDOM
control	94.500			
1.2	92.000	0.120	1.94	k= 1, v= 6
2.0	84.750	0.467	2.06	k= 2, v= 6
3.7	84.750	0.467	2.10	k= 3, v= 6
7.4	84.750	0.467	2.12	k= 4, v= 6
15	84.750	0.467	2.13	k= 5, v= 6

s = 20.895

Note: df used for table values are approximate when v &gt; 20.

%hatch 8 days

File: 5401h8 Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	5.500	5.500	14.500
2	1.2	2.500	2.500	13.500
3	2.0	0.000	0.000	9.000
4	3.7	6.500	6.500	15.500
5	7.4	19.000	19.000	16.500
6	15	0.000	0.000	9.000

Calculated H Value = 2.886      Critical H Value Table = 11.070  
 Since Calc H < Crit H FAIL TO REJECT Ho: All groups are equal.

%hatch 8 days

File: 5401h8      Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

		GROUP			
		TRANSFORMED	ORIGINAL	0 0 0 0 0	
GROUP	IDENTIFICATION	MEAN	MEAN	3 6 2 1 4 5	
3	2.0	0.000	0.000 \		
6	15	0.000	0.000 . \		
2	1.2	2.500	2.500 .. \		
1	control	5.500	5.500 ... \		
4	3.7	6.500	6.500 .... \		
5	7.4	19.000	19.000 ..... \		

\* = significant difference (p=0.05)

Table q value (0.05,6) = 2.936

. = no significant difference

SE = 3.030